

Characterization of allergenic components from house dust mite *Dermatophagoides siboney*. Purification of Der s 1 and Der s 2 allergens

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Summary

Background Allergic reactions to house dust mites of the genus *Dermatophagoides* play an important role in the pathogenesis of asthma and other atopic diseases. *Dermatophagoides siboney* has been described as a species from Cuba. Together with *D. pteronyssinus* and *Blomia tropicalis*, it is frequently found in house dust from homes of asthmatics.

Objectives The aim of this study was to investigate the allergenic composition from the house dust mite *D. siboney*.

Methods The characterization of *D. siboney* extract was performed by SDS-gPAGE and immunoblotting. Purification of individual components was performed by affinity chromatography.

Results At least 16 components between 13 and 98 kDa stained by Coomassie Blue were found. Using a panel of 35 sera from atopic mite sensitive patients 13 components reacted to different extent with patient IgE. Two components, 25 and 14 kDa, bound to specific IgE strongly and frequently, i.e. 80 and 91% of the patients, respectively. Affinity chromatography using crossreacting monoclonal antibodies to group 1 and 2 allergens resulted in purified preparations of 25 and 14 kDa proteins, which showed IgE-binding with the majority of the human sera when tested by immuno-dot.

Conclusion Based on the IgE binding profile of *D. siboney* and on the capacity to react with crossreacting monoclonal antibodies for groups 1 and 2, it is proposed to name these two allergens, 25 and 14 kDa, Der s 1 and Der s 2, respectively.

Keywords: *Dermatophagoides siboney*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, Der s 1, Der s 2, SDS-PAGE, monoclonal antibodies

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Introduction

Allergic reactions to *Pyroglyphid* house dust mites of the genus *Dermatophagoides* play an important role in the pathogenesis of asthma and other atopic diseases. Several species of the genus have been described: *Dermatophagoides pteronyssinus*, *D. farinae*, *D. evansi*, *D.*

microceras, *D. neotropicalis* and *D. siboney*, the latter two from South America and the Caribbean, respectively [1].

Dermatophagoides pteronyssinus and *D. farinae* have been extensively studied as major sources of allergens in house dust from all over the world. The major allergens from both species have been identified and characterized [2–9], and the molecular structure has been studied by monoclonal antibody (MoAb) mapping [10,11], N-terminal sequencing [12–14] and cDNA cloning and expression [15–19].

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As many as seven groups of allergens from *Dermatophagoides* have been described based on their molecular weights and IgE binding frequency [9]. The group 1, 2 and 3 allergens have been studied most.

The Group 1 allergens are found mainly in mite faeces. They are glycoproteins with a molecular weight of 25 kDa. The Group 1 allergens have a cysteine protease activity. They react with specific IgE in more of 80% of sera from mite sensitive patients. The Group 2 allergens have a molecular weight of 14 kDa and are thought to be lysozymes [9,20]. They react to a similar degree with patient sera as Group 1 allergens do. Group 3 allergens have a molecular weight of about 30 kDa and enzymatic activity [9].

Dermatophagoides siboney was described in Cuba in 1982 [21]. *D. pteronyssinus* and *D. siboney* are the most frequent mites in house dust collected from the homes of Cuban asthmatics, i.e. 100 and 85.2%, respectively. Preliminary studies have shown positive skin-test reactions to *D. siboney* in patients with continuous symptoms that indicate possible mite allergy.

The aims of this study were to characterize the allergenic components of *D. siboney* and to purify the major allergens of this species.

Materials and methods

Mite extract

A preparation of *D. siboney* was obtained by extraction of whole mature cultures of mites grown in our laboratory at 25°C and 85% relative humidity on synthetic medium. The cultures have been grown by experienced acarologist which ensure the purity of the species. Extraction was performed at 1:20 (w/v) in ammonium bicarbonate (0.125 M, pH 7.8), for 24 h at 4°C. After centrifugation the supernatant was concentrated by ultra filtration (cut-off 5 kDa, OmegaCell, Filtron, USA), aliquoted and freeze-dried. The aliquots were stored at -20°C.

Sera

Thirty-five sera were collected from 150 adult Cuban asthmatic subjects, during an epidemiological study. They showed continuous symptoms, indicating possible house dust mite allergy and they responded positively by skin-prick tests to a panel of mite allergens (*D. pteronyssinus*, *D. farinae*, *D. siboney*, *A. siro*, *L. destructor* and *B. tropicalis*). Once the sera were obtained they were aliquoted in 2.5 mL fractions, freeze-dried and stored at -20°C. Immediately before use they were reconstituted by adding 2.5 mL of distilled water.

SDS-gPAGE and immunoblot analysis

Freeze-dried allergens were separated by sodium dodecyl sulphate gradient poly-acrylamide gel electrophoresis (SDS-gPAGE) (8–25% gels) under non-reducing conditions by Phast-System (Pharmacia, Sweden). Immediately after electrophoresis, the gels were transferred to 0.2 µm nitro-cellulose membranes (Pharmacia) by semi-dried transfer procedure. Membranes were cut into strips, blocked 30 min at 37°C with PBS buffer, pH 7.3, containing 0.5% Tween 20, and incubated overnight at room temperature with individual patient sera diluted 1:1. After incubation with sera, the strips were washed. Each strip of the membrane was then incubated with ¹²⁵I-anti IgE (Phadebas RAST[®], Pharmacia) (80 000–200 000 cpm per strip) overnight at room temperature. The next day, membranes were extensively washed, mounted into a Kodak X-Omatic cassette with intensifying screen (Eastman Kodak, USA) and autoradiographed on Hyperfilm MP (Amersham, Sweden) during 96 h at -70°C. In each gel transferred, one of the strips contained molecular weight markers (14–94 kDa, Pharmacia). These membrane strips were cut before blocking and then silver stained. A calibration curve was constructed using the R_f of the standards. The corresponding R_f values of the radiolabeled bands were calculated and the respective molecular weights estimated from the standard curve. The intensity of positively radio stained bands was graded arbitrarily according to a scale of four from weakly positive (grade 1) to very strongly positive (grade 4).

Purification of Der s 1 and Der s 2

Raw-material of *D. siboney* was extracted overnight (1:20, w/v) in phosphate buffered saline (PBS). After centrifugation the supernatant was applied on columns of CNBr-Sepharose 4B (Pharmacia) coupled with MoAb 4C1 of 6D6 crossreacting to the Group 1 and Group 2 allergens, respectively [11,12]. The allergens were eluted by 50% ethylene glycol in 0.005 M glycine pH 10.0. The fractions corresponding to the protein peak at 280 nm were pooled and dialysed extensively against 0.005 M ammonium bicarbonate and then freeze-dried. The purity of the materials was checked by SDS-gPAGE, blotted to nitrocellulose, incubated with a pool of five sera, and autoradiographed as described above.

IgE binding of purified allergens

The capacity of purified *D. siboney* allergens to react with human IgE was checked by dot-blotting, using a Bio-Dot apparatus (BioRad, USA). Freeze-dried preparations of

Der s 1 and Der s 2 were resuspended in PBS buffer, pH 7.4, and applied on 0.2 µm nitro-cellulose membranes (Pharmacia) at 2 µL per well. After drying overnight the membranes were blocked by PBS-Tween and washed 3 × 10 min with 300 µL of NaCl-Tween per well. A total of 50 µL of each of 35 patient sera and two controls was applied diluted 1 : 1 to the wells and incubated overnight at room temperature. After washing, 100 µL of ¹²⁵I-anti IgE (4000 cpm, Phadebas RAST, Pharmacia) was added to each well and incubated overnight. Next day the membranes were washed, dried and autoradiographed as described above.

Results

IgE binding profiles

When separated by SDS-gPAGE under non-reducing conditions and stained with Coomassie Brilliant Blue the *D. siboney* extract showed at least 16 strongly stained components between 13 and 98 kDa. The majority of the protein components were located between 20 and 60 kDa (Fig. 1).

Analysis by western blotting and autoradiography revealed 13 proteins with molecular weight between 14 to 95 kDa which reacted to varying degrees with at least one of the sera from mite allergic patients (Fig. 2). The proteins showing the highest prevalence of reactivity with IgE antibody had molecular weights 14 kDa (91%), 25 kDa (80%), and 30 kDa (31%), respectively (Fig. 3). The 95 and 80 kDa bands reacted with a frequency of 29 and 26%, respectively. A protein of 40 kDa was found to react only weakly with one of the patient sera.

As shown in Figs 2 and 3, the 14 and 25 kDa bands showed the highest prevalence of reactivity and also showed the strongest intensity of IgE antibody binding. Grades 2, 3 and 4 predominated in both cases with a mean of grade 3 for the 14 kDa protein and of grades for the 25 kDa protein. Only one patient reacted to the 25 kDa protein without any reaction to the 14 kDa protein, whereas five patients showed IgE binding to the 14 kDa protein without any binding to the 25 kDa protein.

Control sera from patients not sensitized to mites showed no reactivity to *D. siboney* extract.

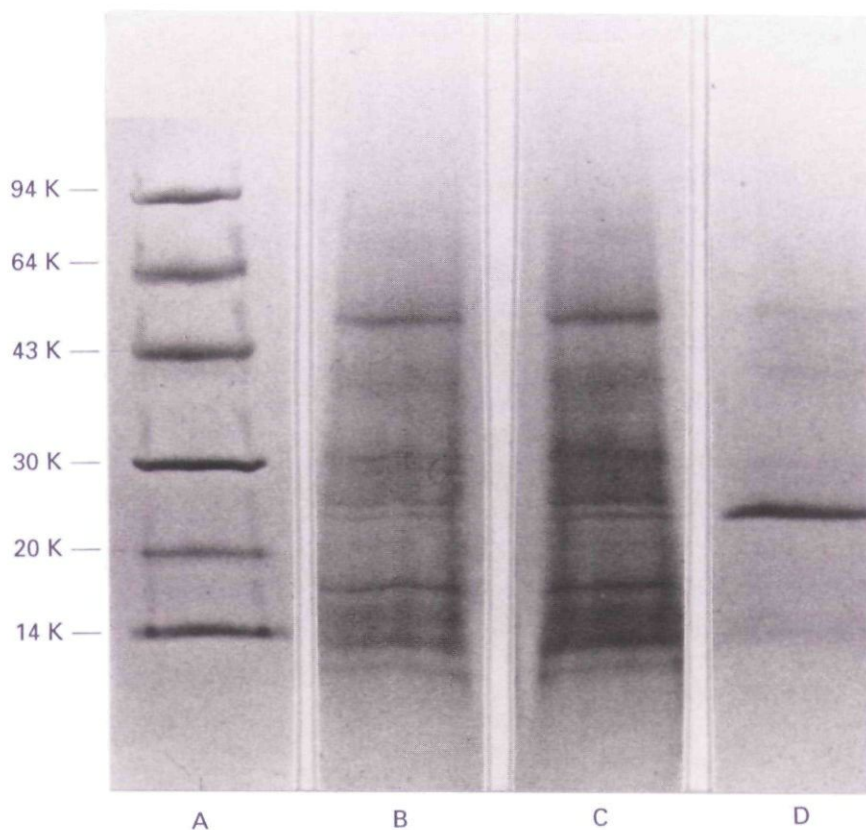


Fig. 1. SDS-gPAGE of *D. siboney*. Lane A: molecular weight markers; Lanes B and C: whole extracts from *D. siboney*; Lane D: affinity chromatography purified Der s 1.

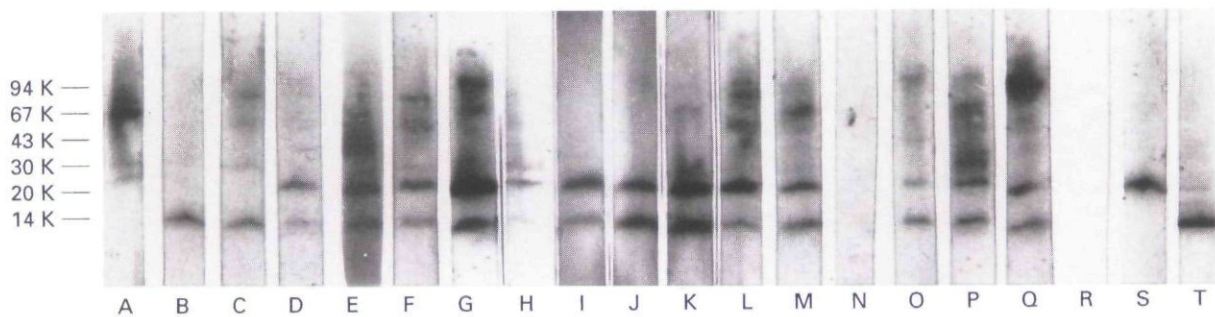


Fig. 2. IgE binding profile of *D. siboney* developed by autoradiography. Lanes A–Q: 17 different selected sera from mite sensitive patients; Lane R: negative control serum; Lane S and T: purified Der s 1 and Der s 2 reacting with a pool of five sera.

Mol. wt.		14 000	19 000	25 000	30 000	37 000	40 000	43 000	52 000	57 000	62 000	65 000	80 000	95 000
Serum number	21	•••		•			•							••••
	22	•••		••••										
	23	•••		•••										
	24	••		•										
	25	•••		••										
	26			•	•						•••		••	•
	27	•••												
	28	•••			••						•••		•••	
	29	••		••••										
	30	•••		••••				••••	•••		••			••
	31	••												
	32	••		••••										
	33	•••		••••	••				•••				•••	
	34	•••		•••	•••									
	35	••••		••••										
	36	••••		••••	••							••		•••
	37	•••	•		••							••		•••
	38	••••		•		•				•			•	
	39	••		•••	••	••				•			•	
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	41	••••		••••						•			•	
	42	••••		••••	••					••			•	
	43	•••		••••	••			•••			••	•••	••••	
	44	•												
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	46	•		•••							••	•••		
	47	••••		••••				•						
	48	••		••										
	49	•••		•										
	50	•••		••••	•								••••	•
	51			No bands found										
	52	•••		••				••						•••
	53	•		••										•
	54	••••		••••	•••			•				•••		•••
	55	•••		•••								••		••••
Neg. Control 1		No bands found												
Neg. Control 2		No bands found												

Fig. 3. Schematic representation of IgE binding profile of *D. siboney* in 35 sera and two negative controls. Dots represent and arbitrary scale of four degrees to represent the difference in the autoradiography intensity for each component.

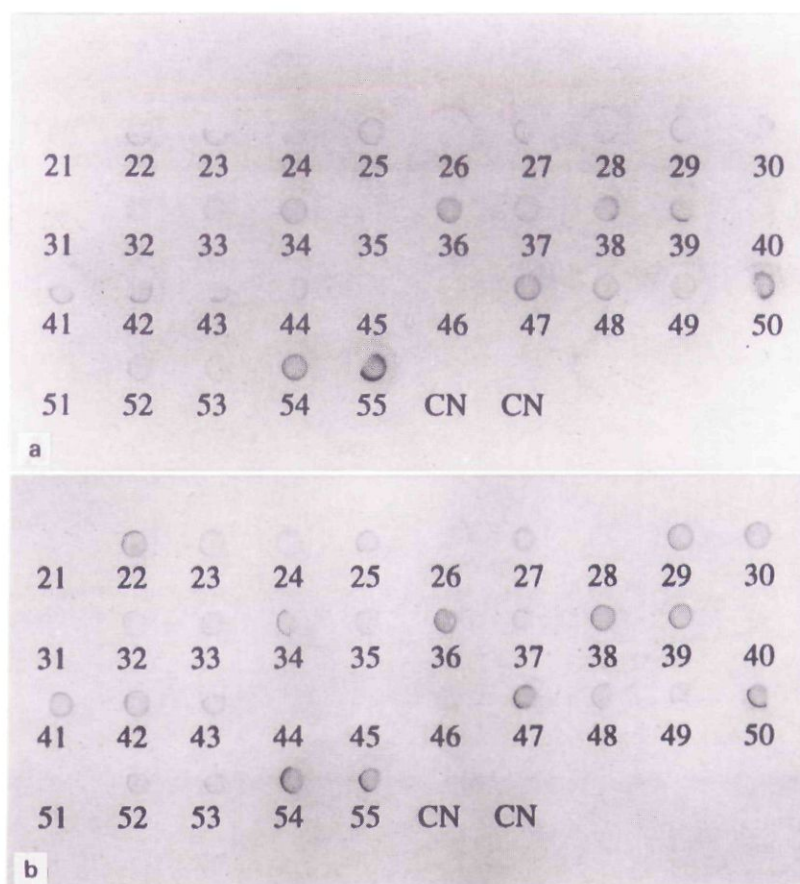


Fig. 4. Immuno-dot analysis of purified Der s 1 (a) and Der s 2 (b) against 35 individual sera and two negative controls (CN).

Purification of *D. siboney* allergens

Affinity chromatography purification of the Group 1 and 2 allergens from *D. siboney*, using 4C1 and 6D6 MoAbs, respectively, resulted in well defined protein peaks that showed a high degree of purity when analysed by SDS-gPAGE (Fig. 1), blotted and autoradiographed (Fig. 2). The purified allergens had molecular weights corresponding to those of Group 1 and 2 allergens, respectively, and reacted strongly with IgE of selected patient sera. The 14 kDa preparation did not show any distinct band when separated by electrophoresis and stained by Coomassie Blue, but when blotted and autoradiographed, then a clear, unique and strong band was observed. Attempts to obtain a more distinct band in SDS electrophoresis by precipitation with 50–80% saturated ammonium sulphate of the affinity purified 14 kDa protein [22], did not improve the appearance of the band. A similar phenomena was observed with the 95 kDa in the extracts. The lack of any distinct band with the purified 14 kDa protein and the 95 kDa protein in the extract, when stained by Coomassie Blue, was presumably due to a low protein content of individual bands.

IgE binding of purified allergens

Both, *D. siboney* Group 1 and 2 purified allergens reacted well with the mite-sensitive sera when tested by immuno-dot. The 25 kDa protein reacted with 31 individual sera, i.e. 89%. Group 2 allergen (14 kDa protein) reacted with sera representing a 86% of IgE binding (Fig. 4).

In general, sera which did not react, or which showed only a weak reaction with both, the 25 kDa and 14 kDa bands in western-blotting experiments, resulted in no or only slight staining in the immuno-dot experiment. With the exception of serum number 35 for 25 kDa protein and sera 21 and 31 for 14 kDa protein, results of immuno-dot agreed with those obtained by western-blotting. The different amounts of proteins transferred onto the membranes, could explain the minor differences observed between experiments. No reactions were found with sera from healthy controls in these experiments.

Discussion

Dermatophagoides siboney is closely related to *D. farinae* and *D. microceras*, from which it differs mainly in the

markedly smaller dimensions of body and setae [21], it is commonly present in house dust in Cuba. Together with *D. pteronyssinus* and *B. tropicalis*, *D. siboney* is an important source of mite allergens in Cuba.

It is well known that major allergens of different members of the genus *Dermatophagoides*, especially *D. pteronyssinus* and *D. farinae* are very similar [3,10,12,19,23] and that there are morphological similarities between the six reported domestic species of the genus [1,21].

The IgE binding profile of *D. siboney* agrees with that previously found for other members of the genus [2–5]. In agreement with Shibasaki *et al.* for *D. farinae* [24], we found 13 IgE binding components in this extract of *D. siboney*, which is less than reported for *D. pteronyssinus* [2,5] and *D. farinae* [25]. These figures, however, depend to some extent upon the sera tested as well as on the raw material used for the preparation of extracts. Sera from patients with relatively low levels of anti-mite specific IgE antibodies and small skin-test reactions (i.e. sera 24, 31, 44 and 51; data not shown) gave no or only weak binding of IgE to the bands in SDS-gPAGE and in the immunodot experiments.

The high percentage of sensitive patients who showed IgE against the 14 and 25 kDa proteins from *D. siboney*, define these proteins as major allergens. The molecular weights of these *D. siboney* allergens agreed with those of other Group 1 and Group 2 *Dermatophagoides* allergens.

Moreover, crossreacting MoAbs directed against epitopes on both *D. pteronyssinus* and *D. farinae* were used for purification of *D. siboney* allergens. We checked by immunoblotting (data not shown) the reaction of the monoclonal antibodies used for purification (anti-group 1: 4C1 and anti-group 2: 6D6) against the extract of *D. siboney*. The results agree with those obtained with human sera, and the 14 kDa and 25 kDa bands were also identified on the membranes of the crude extract when developed by anti-mouse Ig(s)-HRP conjugate and DAB substrate. According to the WHO/IUIS allergen nomenclature, we propose to name the 25 kDa protein, which reacted with 80% of sera, Der s 1, and the 14 kDa protein, which reacted with more than 90% of sera, Der s 2.

A higher prevalence of IgE antibody binding was found with Der s 2 than with Der s 1, in keeping with previous reports for *D. pteronyssinus* [2] and *D. farinae* [24]. The fact that we used whole culture extract containing not only mite-faecal material but mite bodies, could explain these results.

More than 30% of the tested sera reacted with a 30 kDa protein, which might represent the *D. siboney* group 3 allergen. There are differing reports on the IgE reactivity of Group 3 allergens [19]. The frequency of IgE

binding found in western blotting experiments was lower than reported by some authors for Der p 3 or Der f 3 [2,5,9,13], but higher than found by others [12]. According to Yasueda *et al.* [13] the variation in frequency of IgE antibodies against the Group 3 allergens may be due to differences in purity of the allergen preparations used in the populations studied or in the methods for measurement of specific IgE anti-Group 3. This may also be true for the differences in binding profile between studies. The use of higher concentrations of mite extracts as well as more concentrated sera in blotting experiments might result in a higher frequency of binding Group 3 allergens. To confirm its identity as Der s 3, the protein should be purified and compared to previously described Group 3 allergens by testing its binding to 5A12 Der Group 3 MoAb [12].

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References

- Colloff MJ, Spieskma FTM. Pictorial keys for the identification of domestic mites. *Clin Exp Allergy* 1992; 22:823–30.
- Baldo BA, Ford SA, Tovey ER. Toward a definition of the complete spectrum and rank order of importance of the allergens from the house dust mite: *Dermatophagoides pteronyssinus*. *Adv Biosciences* 1989; 74:13–31.
- Lind P. Demonstration of close physicochemical similarity and partial immunochemical identity between the major allergen, Dp-42, of the house dust mite, *D. pteronyssinus* and corresponding antigens of *D. farinae* (Df6) and *D. microceras* (Dm6). *Int Archs Allergy Appl Immunol* 1986; 79:60–5.
- Lind P, Lowenstein H. Identification of allergens in *Dermatophagoides pteronyssinus* mite body extract by crossed radio-immunoelectrophoresis with two different rabbit antibody pools. *Scand J Immunol* 1983; 17:263–73.
- Tovey ER, Baldo BA. Comparison by electroblotting of IgE-binding components in extracts of house dust mite bodies and spent mite culture. *J Allergy Clin Immunol* 1987; 79:93.
- Lind P. Characterization of a partially purified allergen preparation of *Dermatophagoides pteronyssinus* and of 2 isolated major allergens (abstract). 14th Nordic Congr Allergology, Gothenburg, 1981, p. 24.
- Lind P. Purification and partial characterization of two

- major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1985; 76:753.
- 8 Platts-Mill TAE, Thomas WR, Aalberse RC, Vervloet D, Chapman MD. Dust mite allergens and asthma: Report of a second international workshop. *J Allergy Clin Immunol* 1992; 89:1046–60.
 - 9 Thomas WR. Mite allergens groups I–VII. A catalogue of enzymes. *Clin Exp Allergy* 1993; 23:350–3.
 - 10 Chapman M, Heymann PW, Platts-Mills TAE. Epitope mapping of two major inhalant allergens, Der p I and Der f I, from mites of the genus *Dermatophagoides*. *J Immunol* 1987; 139:1479–84.
 - 11 Le Mao J, Weyer A, Mazie JC et al. Identification of allergenic epitopes on Der f I, a major allergen of *Dermatophagoides farinae*, using monoclonal antibodies. *Mol Immunol* 1992; 29:205–11.
 - 12 Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TAE. Antigenic and structural analysis of group II allergens (Der f II and Der p II) from house dust mites (*Dermatophagoides* spp.). *J Allergy Clin Immunol* 1989; 83:1055–67.
 - 13 Yasueda H, Mita H, Akiyama et al. Allergens from *Dermatophagoides* mites with chymotryptic activity. *Clin Exp Allergy* 1993; 23:384–90.
 - 14 Stewart GA, Thompson PJ, Simpson RJ. Protease antigens from house dust mite. *Lancet* 1989; ii:154–5.
 - 15 Yuki T, Okumura Y, Ando T et al. Cloning and expression of cDNA coding for the major house dust mite allergen Der f II in *Escherichia coli*. *Agric Biol Chem* 1991; 55:1233–8.
 - 16 Dilworth RJ, Chua KY, Thomas WR. Sequence analysis of cDNA coding for a major house dust mite allergen, Der f I. *Clin Exp Allergy* 1991; 21:25–32.
 - 17 Trudinger M, Chua KY, Thomas WR. cDNA encoding the major mite allergen Der f II. *Clin Exp Allergy* 1991; 21:33–7.
 - 18 Shen HD, Chua KY, Lin KL, Hsie KH, Thomas WR. Molecular cloning of a house dust mite allergen with common antibody binding specificities with multiple components in mite extracts. *Clin Exp Allergy* 1993; 23:934–40.
 - 19 Smith WA, Chua KY, Kuo MC, Rogers BL, Thomas WR. Cloning and sequencing of the *Dermatophagoides pteronyssinus* group III allergen, Der p III. *Clin Exp Allergy* 1994; 24:220–8.
 - 20 Stewart GA, Bird CH, Thompson PJ. Do the group II allergens correspond to lysozyme? *J Allergy Clin Immunol* 1992; 90:141–2.
 - 21 Dusbabek F, Cuervo N, De la Cruz J. *Dermatophagoides siboney* sp.n. (Acarina: Pyroglyphidae) a new house dust mite from Cuba. *Acarologia* 1982; 23:55–62.
 - 22 Lombardero M, Heymann PW, Platts-Mills TAE, Fox JW, Chapman MD. Conformational stability of B cell epitopes on group I and group II *Dermatophagoides* spp. allergens. Effect of thermal and chemical denaturation on the binding of murine IgG and human IgE antibodies. *J Immunol* 1990; 144:1353–60.
 - 23 Heyman PW, Chapman MD, Platts-Mills TAE. Antigen Der f I from the dust mite *Dermatophagoides farinae*: structural comparison with Der p I from *D. pteronyssinus* and epitope specificity of murine IgG and IgE antibodies. *J Immunol* 1986; 137:2841–7.
 - 24 Shibasaki M, Isoyama S, Takita H. Influence of age on IgE responsiveness to *Dermatophagoides farinae*: An immunoblot study. *Int Arch Allergy Immunol* 1994; 103:53–8.
 - 25 Nakanishi K, Shimokata K. Immunoblot analysis of *Dermatophagoides farinae* antigen. *Ann Allergy* 1990; 64:219–23.

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